INHERITANCE OF RESISTANCE TO LOOSE SMUT, *USTILAGO NUDA*, IN BARLEY

William P. Skoropad

Department of Plant Science
University of Alberta

THE UNIVERSITY OF ALBERTA

INHERITANCE OF RESISTANCE TO LOOSE SMUT, *USTILAGO NUDA*, IN BARLEY

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT SCIENCE

by

William P. Skoropad

EDMONTON, ALBERTA

AUGUST, 1951

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

ABSTRACT

The inheritance of resistance to two distinct races of loose smut, "Tr." and "Ts." was studied in several crosses and backcrosses involving barley varieties Jet and Titan as resistant parents. Both Jet and Titan are resistant to race Tr., but Titan is susceptible to Ts.

Classification of F_2 plants, based on the behavior of their progenies in F_2 rows and the F_3 progeny tests of backcrossed plants formed the basis for the genetical analysis. Averages of 80 lines in the F_1 and 180 lines in the F_3 generations were used. A single dominant gene was found to govern the resistance of Jet and Titan to race Tr. The resistance of Jet to Ts. was conditioned by an additional gene which has the supplementary capacity of increasing resistance to Tr. Resistance was found to be determined by the genetic constitution of the developing embryo rather than that of the surrounding floral tissue.

INHERITANCE OF RESISTANCE TO LOOSE SMUT, USTILAGO NUDA, IN BARLEY

William P. Skoropad

INTRODUCTION

Loose smut of barley is caused by *Ustilago nuda* (Jens.) Rostr., a floral infecting fungus which produces a deep internal infection of the seed. The disease causes reductions in yield approximately equivalent to the percentage of smutted plants (11). A modified hot-water treatment (1) for destroying the dormant mycelium of *Ustilago nuda* is difficult to apply and even under carefully controlled conditions, may cause some damage. Schafer and Hansing (9) reported that variety, season and location influenced the amount of injury to barley seed treated by this method. These factors complicate the practical control of loose smut in susceptible varieties of barley. Breeding and distributing resistant varieties, therefore, offers the best means of control.

An understanding of how resistance is inherited is important in a breeding program of this nature. Resistance controlled by a single gene is more readily utilized than that of a complex nature.

In a series of crosses Zeiner (14) found indications that single genes conditioned immunity, resistance, and moderate susceptibility. Resistance appeared to be dominant over susceptibility. Livingston (2) reported the presence of a single dominant factor for resistance in Trebi and in a selection of Hordeum deficiens. Schaller (10) confirmed Livingston's findings concerning Trebi and demonstrated single gene resistance in varieties Jet, Dorsett and selection X173-10-5-6-1. In keeping with the recommendations of Robertson, Wiebe and Shands (4), he suggested the symbols Un_3, Un_4, and Un_5 for genes in Jet, Dorsett and selection X173-10-5-6-1, respectively. He also found the Trebi gene to be independent of the Jet and Dorsett genes.

Until 1946, barley breeders at the University of Alberta used the Titan variety extensively as a resistant parent in their breeding program. In that year a higher infection rate was observed in Titan and has subsequently increased to a point where the variety must be classed as susceptible. This change in resistance of Titan to loose smut

suggested the appearance of a new race of Ustilago nuda.

Physiological specialization has been shown to occur within the loose smut organism (5, 6, 8, 13), making it desirable to have more than one available source of resistance. Rodenhiser (6) reported twelve physiologic forms of Ustilago nuda. Thren (13) stated that the chlamydospores of Ustilago nuda usually germinate so that nuclear pairing takes place in the promycelial stage and therefore this almost complete elimination of a haploid phase reduces the chance of out-crossing and is correlated with the low degree of variability, the low mutation frequency and the few races of this species.

OBJECTIVES

The objective of this project is to determine the mode of inheritance of resistance to: (a) the original race of loose smut to which Titan is resistant, and (b) the new race to which Titan is susceptible.

This paper deals with the genetics of resistance to two different races of loose smut in two varieties of barley.

Varieties were selected which differed in their level of resistance to the new and the old race of loose smut.

MATERIAL AND METHODS

Since two different races of loose smut were involved in this project it was necessary to differentiate them by some simple method. The new race has not been named yet and therefore in this paper the following designations are used: Tr for the race to which Titan is resistant and Ts for the race to which Titan is susceptible.

Hybrid populations from crosses of the various combinations of Jet, Montcalm, Sanalta and Titan were used in this investigation. The reaction of these varieties to the Tr and Ts races of loose smut are shown in Tables I and II respectively.

Table I

Reactions of parental varieties when inoculated artificially with Ustilago nuda, race Tr.

Variety	Tests* Number	Range of infection per cent	Average infection per cent
Jet	108	0	0.00
Montcalm	117	55 - 80	68.32
Sanalta	98	65 - 90	82.56
Titan	130	0 - 4.5	1.11

* A test denotes the progenies from three inoculated heads.

Table II

Reactions of parental varieties when inoculated artificially with Ustilago nuda, race Ts.

Variety	Tests* Number	Range of infection per cent	Average infection per cent
Jet	95	0	0.00
Montcalm	85	65 - 85	78.00
Sanalta	92	70 - 95	92.76
Titan	173	25 - 50	45.38

* A test denotes the progeny from three inoculated heads.

Jet, in which no smutted plants have been found, is a black, naked-kerneled, 2-rowed variety of Abyssinian origin. It is of no commercial importance in Canada but is valuable as a source of resistance in breeding programs designed to develop varieties resistant to loose smut.

Titan is a white, 6-rowed, smooth-awned variety. It is very strong strawed and strong necked and is an important feed barley in Alberta. It was developed by the University of Alberta from a Trebi x Glabron cross. It is highly resistant to *Ustilago nuda*, race Tr. but susceptible to *Ustilago nuda*, race Ts.

Montcalm is a white, 6-rowed, semi-smooth variety. It was developed from a cross (Michigan 31604 x Common Six-Rowed 4307 M.C.) x Manscheuri 1807 M.C.

The Michigan 31604 parent is a black, smooth-awned variety, while the other two parents are of the Manchuria type. It was produced at Macdonald College, Quebec, and released in 1945. Montcalm is the standard of malting barley in Canada. It is susceptible to both races of loose smut.

Sanalta is a white, smooth-awned, two-rowed variety. It came from a cross Smooth Awn x Duckbill and was produced at the University of Alberta, Edmonton, Alberta. It was released in 1940. Sanalta is grown in small areas scattered

throughout Canada, becoming popular in Manitoba and Alberta. It is highly susceptible to both races of loose smut.

The "hypodermic" method of inoculation as described by Poehlman (3) was used throughout this investigation. A rubber bulb containing an aqueous suspension of about three per cent by volume of chlamydospores of Ustilago nuda was attached to a hypodermic needle (20 gauge). The two lower and the two top florets of a barley head were removed with forceps because they were generally found to be late in maturing. The awns were clipped to facilitate the operation of the needle. A drop of this chlamydospore suspension was injected into each floret by piercing the lemma with the needle and squeezing the bulb slightly. Under greenhouse conditions the only modification in the technique was that the excess water was sucked back from the floret into the rubber bulb.

Inoculations were made at a stage between pollen dehiscence to shortly after fertilization. Other workers (2, 10, 12, 7) found this to be the period at which the ovary is most susceptible to infection. The plants from the inoculated seed were grown mostly in the field. There was no difference in the rate of infection of plants by loose smut when the field grown plants were compared with those grown in the greenhouse.

The supply of inoculum of the Ts. race was built up and maintained on Titan while the Tr. race inoculum was maintained on Montcalm. The identity of each race of smut was frequently checked by its reaction on Titan. The inoculated seed was treated with organic mercuric dust before planting to reduce seedling loss and increase vigor.

Hybrid material was tested in F_1, F_2, F_3 and backcross generations. The F_2 generation does not permit a satisfactory genetical analysis since there is likely to be a preponderance of resistant plants possibly due to the lethal effects of infection (14) and the failure to obtain a phenotypic expression of all genetically susceptible plants. Classification of F_2 plants, based on the behaviour of their progenies in F_3 rows, and the F_2 progeny tests of backcrossed plants formed the basis for the genetical interpretation of the genes involved.

The reaction of the F_1 generation was obtained by inoculating the flowers of the female parent one day after the introduction of the pollen from the male parent and then growing the plants from the inoculated seeds.

Tests on F_3 lines were made by inoculating the flowers of two heads of F_2 plants taken at random from a disease-free F_2 nursery. The two heads from each plant were grown separately

and the average infection was used in determining the reaction of the F_2 plant. Parental checks were grown at regular intervals.

The material from the backcross was handled in a similar manner; i.e., flowers on two heads of each backcross F_1 plant were inoculated.

A single smutted tiller in a plant was sufficient to classify it as susceptible. The total number of plants and the number of smutted plants were recorded and the percentage of smut calculated.

In the genetic analysis, probability (P) values were calculated for each hypothesized ratio by the X^2 test of goodness of fit.

EXPERIMENTAL RESULTS

I. SMUT INFECTION IN THE F_1

1. Inoculation With Race Tr.

In order to expose the progeny of a hybrid barley plant to infection by Ustilago nuda, the heads were inoculated at flowering time. The tissues through which the infecting hyphae must penetrate to the ovary are those of the parent plant, which may differ genetically from those of the embryo to be tested for susceptibility. It was necessary, therefore, to ascertain whether the results of inoculation were influenced by the parent-plant tissue or by the embryo.

The smut reactions of the F_1 generation of the various crosses and their reciprocals artificially inoculated with race Tr. are shown in Table III.

Table III

Infection of F_1 barley hybrids inoculated with race Tr.

Hybrid	Total plants Number	Infected Plants Number	Infected Plants Per cent
Sanalta x Jet	55	0	0.00
Jet x Sanalta	42	0	0.00
Sanalta x Titan	84	2	2.38
Titan x Sanalta	106	2	1.89
Montcalm x Jet	163	0	0.00
Jet x Montcalm	87	0	0.00
Montcalm x Titan	93	1	1.08
Titan x Montcalm	84	0	0.00
Titan x Jet	55	0	0.00

The amount of infection for reciprocal crosses was the same indicating that using any variety as a male or female parent had no different influence on the amount of infection by the loose smut organism.

The absence of infection in the F_1 generation of all crosses involving Jet must be attributed to the heterozygous

embryo, as the floral tissue of the female parents, Sanalta and Montcalm was susceptible.

The 2.14 per cent average smut infection in the F_1 of Sanalta x Titan and its reciprocal is only slightly higher than the smut infection of the resistant parent Titan (Table I). This is probably due to the small number of plants used and, therefore, it may be considered that Titan exercised its full resistance in the F_1 hybrids involving Sanalta. F_1 hybrids of which Montcalm and Titan ~~are~~ were parents gave an average infection of 0.54 per cent and this rate, too, is in fairly good agreement with the infection range of Titan (Table I). Combining the infection rates of Titan with Montcalm and Sanalta as well as their reciprocals, an average infection rate of 1.34 per cent which is in very good conformity with the percentage of smut in Titan.

Resistance to infection by Tr. must, therefore, be determined by the genetic constitution of the developing embryo rather than that of the surrounding floral tissue.

2. Inoculation with Race Ts.

The smut reactions of the F_1 generation of the various crosses and their reciprocals artificially inoculated with *Ustilago nuda* race Ts. are shown in Table IV.

Table IV

Infection of F_1 barley hybrids inoculated with race Ts.

Hybrid	Total plants Number	Infected Plants Number	Infected Plants Per cent
Sanalta x Jet	66	0	0.00
Jet x Sanalta	188	0	0.00
Sanalta x Titan	88	50	56.82
Titan x Sanalta	123	68	55.28
Montcalm x Jet	156	0	0.00
Jet x Montcalm	96	0	0.00
Montcalm x Titan	74	39	52.70
Titan x Montcalm	108	60	55.56
Titan x Jet	177	0	0.00

The amount of infection for the reciprocal crosses is the same indicating that in this respect Ts. acted similarly to Tr. This also confirms the fact that regardless of the genetical nature of the floral tissue, the amount of infection is the same.

Also similar to the reaction of the hybrids to Tr. is the complete absence of infection in the F_1 hybrids having Jet as one of the parents. The resistance of Jet must be completely dominant and hence exercises its influence to maintain immunity to loose smut even when in heterozygous form.

Crosses of Titan x Sanalta and its reciprocal gave an average infection rate of 56.05 per cent. This rate is somewhat higher than the average infection for the more resistant parent, Titan (Table II), but is considerably lower than the average infection for Sanalta (Table II).

Crosses of Titan x Montcalm and their reciprocal follow the same general trend as the F_1 lines of Titan x Sanalta; i.e., the average infection rate of 54.13 per cent is somewhat higher than the average for the more resistant parent, Titan (Table II), but far below the infection rate for Montcalm. It may be suggested that Titan failed to keep its F_1 progeny with Sanalta and Montcalm down to its own level of resistance because of incomplete dominance of its gene for resistance. There is a possibility, too, that the populations used in these tests were too small and that appreciably different results would be obtained by increasing them.

In F_1 barley hybrids inoculated with either Tr. or Ts. there are strong indications that resistance to the loose smut organism is determined by the nature of the embryo, rather than by the resistance or susceptibility of the floral tissues of the female parent on which they are borne. This means that in F_1 plants the hyphae of the chlamydospores of Ustilago nuda race Tr. or Ts. were able to penetrate tissue possessing a gene for resistance and infect embryos that did not have this gene. Similar results were obtained by Schaller (10) and Livingston (2).

II. INHERITANCE OF RESISTANCE

1. Observations in the F_1

The amount of infection in F_1 hybrids as shown in Tables III and IV, demonstrates that resistance of Jet and of Titan is Controlled by a ~~transmitted as a~~ dominant gene. ~~character~~. Since in all hybrids involving Jet there was not a single smutted plant,

it may be concluded that resistance in Jet is completely dominant. In hybrids having Titan as one of the parents and inoculated with race Tr. none of the infection rates were considerably higher than that of the more resistant parent, Titan. While the data are not extensive, there are indications that in this case the resistance of Titan is also completely dominant. Hybrid F_1 material involving Titan and inoculated with race Ts. shows a tendency to a slightly higher infection rate than Titan but considerably lower than the more susceptible parent Sanalta or Montcalm. From this information it may be argued that although the relative resistance of Titan is dominant, it is not completely dominant to the Ts. inoculum.

2. F_2 Data on Resistance to Race Tr.

(a) Reaction to race Tr.

Since two different races of loose smut were used in this study it will be necessary to discuss results of each set of inoculations separately and hence for the F_2 and F_3 generations they are listed under separate headings.

Infection data for the F_2 generation are given in Table V.

Table V

Infection of F_2 barley hybrids inoculated with chlamydospores of race Tr.

Hybrid	Total plants Number	Infected Plants Number	Infected Plants Per cent
Sanalta x Jet	282	10	3.55
Jet x Sanalta	214	12	5.61
Total	496	22	4.44
Montcalm x Jet	444	19	4.28
Jet x Montcalm	245	8	3.27
Total	689	27	3.92
Sanalta x Titan	424	44	10.38
Titan x Sanalta	157	21	13.38
Total	581	65	11.19
Montcalm x Titan	226	19	8.41
Titan x Montcalm	204	15	7.35
Titan x Jet	260	0	0.00

The amount of infection for the reciprocal crosses is the same and thus confirms the observation made in the F_1 generation that the embryo and not the maternal tissue is responsible for resistance to loose smut.

The proportion of infected plants in reciprocal crosses of Titan with Montcalm and Titan with Sanalta (Table V) suggests a dihybrid ratio. However, it must be borne in mind that the susceptible parents, Montcalm and Sanalta, did not develop 100 per cent infection when inoculations were made at different periods and with all possible precautions.

In reciprocal crosses of Jet with Sanalta and Jet with Montcalm (Table V) there is even a greater preponderance of healthy plants than in crosses with Titan. This condition is probably attributable to Jet being absolutely resistant to infection by Tr. whereas Titan showed an average infection of 1.11 per cent (Table I).

It is impossible to determine the genetics of resistance to loose smut from the F_2 population because each seed rather than each head serves as a unit ofcomputation and hence reductions in observable infected plants may be quite drastic due to reduced germination of infected seed (14), selective mortality of infected plants as well as selective mortality of flowers after inoculation (2). The main genetical inter-

pretation must, therefore, come from the F_3 and F_2 backcross generations.

The hypothesis that the maternal tissue offers no obstacle to the penetration of the chlamydospore hyphae is further confirmed by the fact that the infection rate rises considerably in the F_2 generation. Floral tissue in this generation was heterozygous resistant.

(b) Inheritance of reaction to race Tr. in Jet hybrids.

Jet was immune while Sanalta was highly susceptible (Table I), when artificially inoculated with Tr. The F_1 generation was resistant when either variety was used as a female parent (Table III). The absence of infection in the F_1 generation and the low amount of infection in the F_2 generation suggested that the resistance of Jet was transmitted as a dominant ~~character~~ gene, and that the infection of hybrids depended on the nature of the embryo rather than the floral tissue of the female plant.

The smut infection of 204 F_3 progenies of Sanalta x Jet by five per cent classes is given in Table VI and shown graphically in Figure 1.

Table VI

Distribution of F_3 and backcross progenies for loose smut infection by percentage classes for crosses artificially inoculated with race Tr.

Crosses	Total No. of rows	Percentage classes: 0	0.1-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	70-75	75-80	80-85	85-90	90-95	95-100
Sanalta x Jet	204	58	18	28	30	14	11	2	0	0	0	0	0	2	1	6	8	5	9	1	7	4
Sanalta x F_1 (Sanalta x Jet)	84		25	4	3	1	1	1	1	0	0	0	2	1	3	7	3	6	14	8	4	
Montcalm x Jet	192	55	32	21	24	6	13	0	2	0	1	2	1	3	9	20	3	0	0	0	0	0
Montcalm x F_1 (Montcalm x Jet)	116		48	6	1	2	3	2	1	2	0	0	3	3	31	2	11	0	1	0	0	0
Sanalta x Titan	216	38	47	31	19	10	5	0	0	2	1	0	7	15	29	10	2	0	0	0	0	0
Sanalta x F_1 (Sanalta x Titan)	64	0	0	12	2	8	2	1	1	0	3	2	5	18	4	6	0	0	0	0	0	0
Montcalm x Titan	166	29	33	26	18	6	10	3	5	1	1	5	9	17	2	1	0	0	0	0	0	0
Titan x Montcalm	184	41	42	35	4	4	4	5	3	1	2	1	4	26	8	1	1	2	0	0	0	0

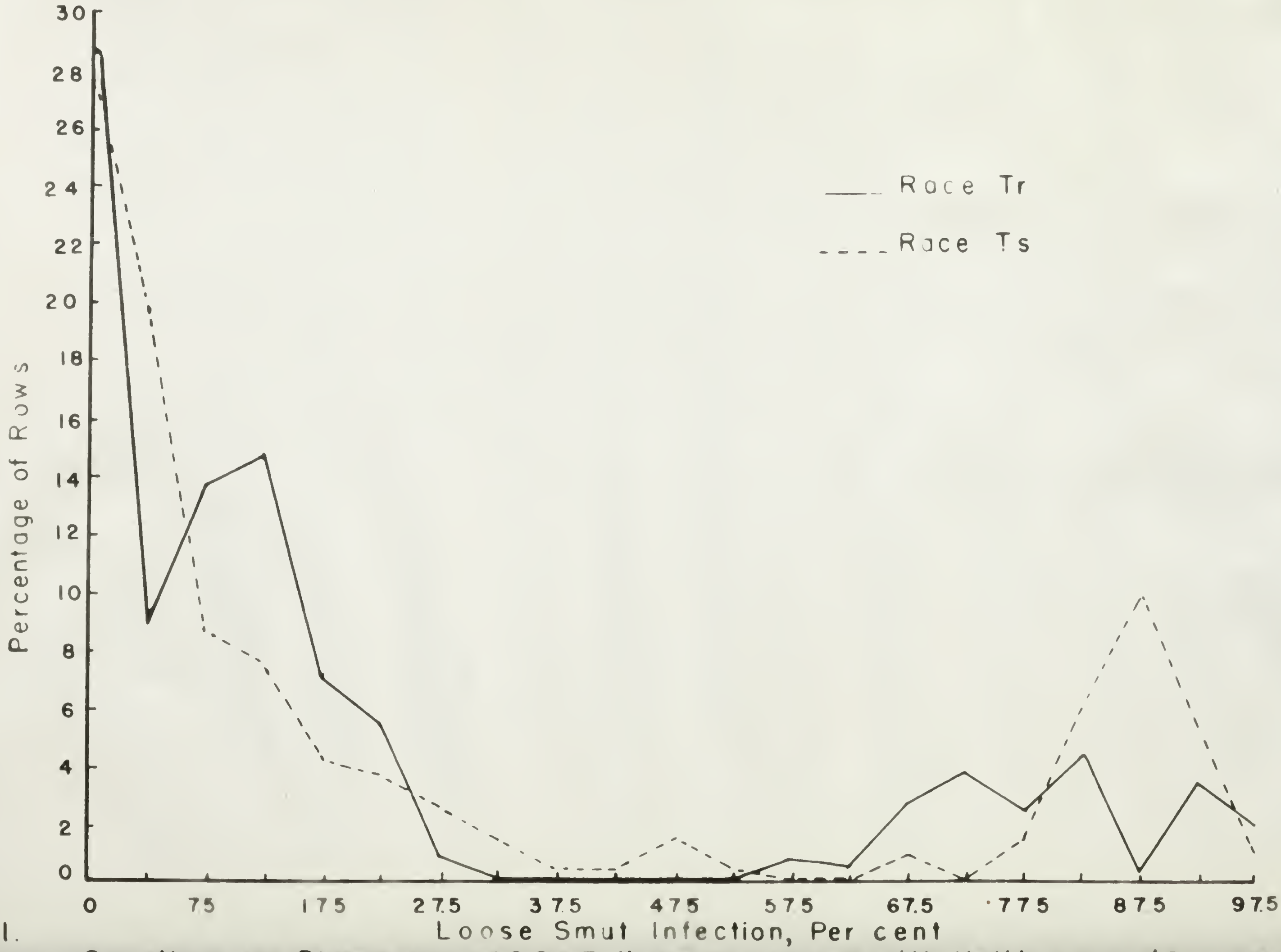

FIG.I.
Sonalta x Jet Distribution of 204 F lines inoculated with Ustilago nuda race Tr

The absence of lines between 32.5 and 52.5 per cent classes divided the progenies into two groups. There were 161 families in the 0 to 32.5 per cent infection range and 43 families in the 52.5 to 97.5 per cent infection group. The progenies of these two groups approximate a 3:1 ratio which would be expected if a single major gene for resistance were acting. The X^2 test for goodness of fit to a 3:1 ratio gave a probability of 0.20 to 0.10.

Jet is absolutely resistant to the chlamydospores of Tr. and therefore it may be assumed that all F_3 lines falling in the 0 per cent infection class must have come from homozygous resistant F_2 plants. Also it is noted that the F_3 lines falling in the high infection group (Table VI) are fairly well within the range of infection of the susceptible parent, Sanalta (Table I) and therefore it may be assumed that this F_3 group was produced from the homozygous recessive F_2 plants. The F_3 group falling in the 1 to 27.5 per cent infection class could, therefore, be considered as coming from the segregating F_2 lines.

On the above basis the F_3 progenies were thus further classified as highly resistant, segregating and highly susceptible. There were 58 F_3 families containing no smutted plants, 103 F_3 families showing an infection range of 1 to 27.5 per cent, and 43 F_3 families having over 52.5 per cent infection. If a single major gene in Jet were responsible for resistance then the above distribution should conform to a 1:2:1 ratio. In testing the

goodness of fit of these data to the above ratio, a P value of 0.50 to 0.30 was obtained. Thus, according to the distribution of F_3 progenies of the cross Sanalta x Jet, it may be concluded that the resistance of Jet is conditioned by a single dominant gene.

A total of 192 families inoculated with race Tr. was grown from a smut-free F_2 nursery of the cross Montcalm x Jet. A distribution of these F_3 families for infection to loose smut is given in Table VI and graphically illustrated in Figure 2. The small number of lines in the 27.5 to 52.5 per cent infection class splits the progenies into two groups with 153 families showing less than 37.5 per cent smutted plants and 39 families with more than 37.5 per cent infection. A distribution of this kind suggests a 3:1 ratio which would be expected if resistance of Jet to loose smut is controlled by a single dominant genetic factor. A X^2 test for goodness of fit to a 3:1 ratio gave a probability of 0.20 to 0.10, thus confirming the hypothesis that Jet carries a single major gene for resistance to the loose smut organism.

Since Jet is completely resistant it may be assumed that all the F_3 lines showing no infection at all must have come from homozygous resistant F_2 plants. The break in the infection curve for the F_3 progenies of Montcalm x Jet comes at the 37.5

per cent class. The group of families falling in the 37.5 to 72.5 per cent infection class is fairly well within the infection range of the susceptible parent, Montcalm, and hence it may be assumed that these F_3 lines have been grown from homozygous susceptible F_2 plants. Families that fall in the 1 to 37.5 per cent class may, therefore, be classified as arising from heterozygous F_2 plants.

In accordance with the above classification, there were 55 homozygous resistant F_3 families, 98 segregating F_3 families, and 39 homozygous susceptible F_3 families. The above distribution should conform to a 1:2:1 ratio if a single major gene in Jet is responsible for resistance to loose smut. A P value of 0.30 to 0.20 was obtained for the above ratio and hence confirms the above statement concerning the genetical analysis of Jet's resistance to Tr.

The F_1 plants backcrossed to the susceptible parent were grown without inoculation and the lines obtained were tested for loose smut reaction. The data for Sanalta x F_1 (Sanalta x Jet) are presented in Table VI. A complete break at the 32.5 to 52.5 per cent class divides the progeny into two groups of 36 lines with less than 32.5 per cent infection and 48 lines with more than 52.5 per cent infection.

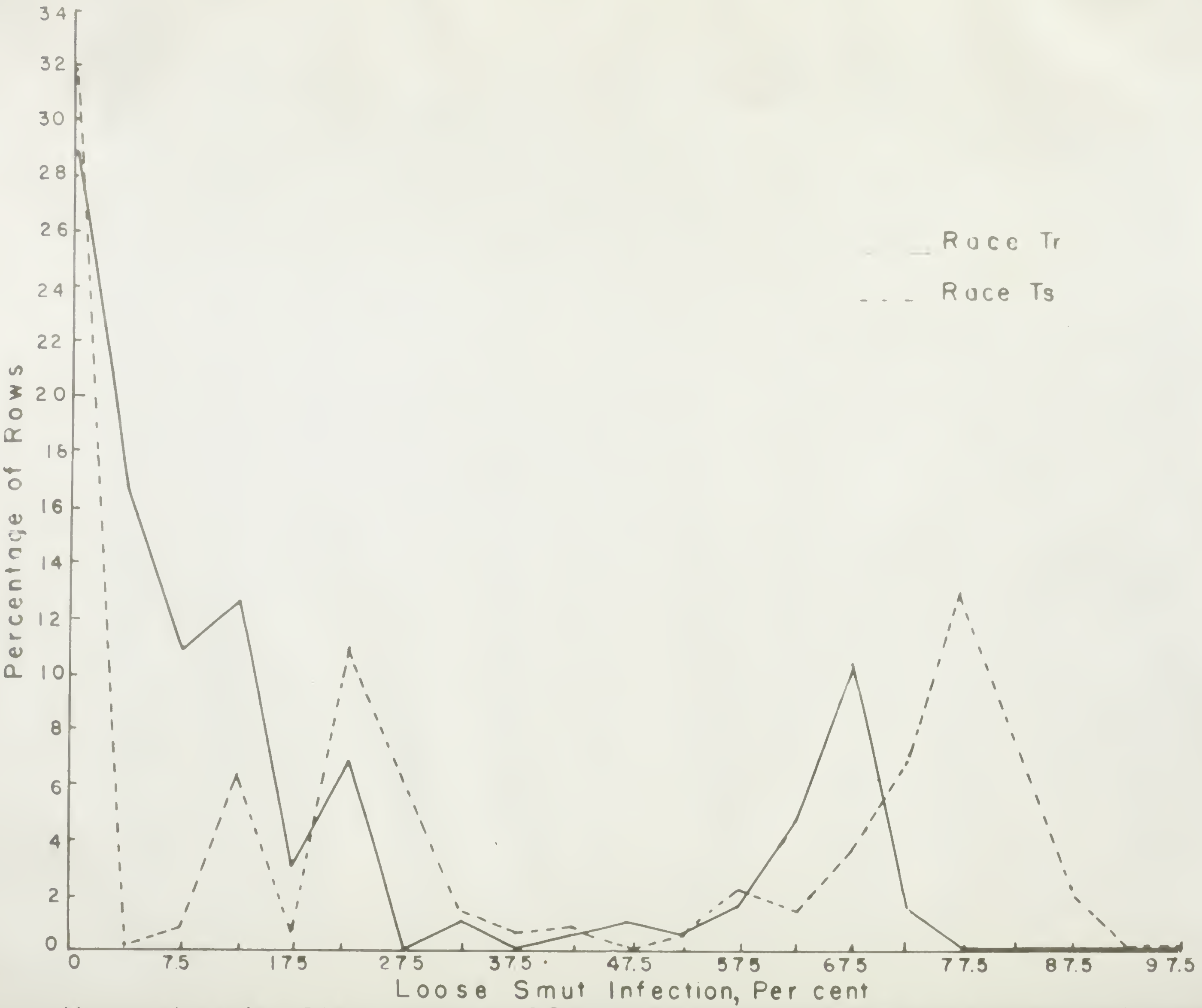

FIG 2.
Montcalm x Jet. Distribution of 192 F_3 lines inoculated with Ustilago nuda, race Tr

The above distribution should conform to a 1:1 ratio if resistance of Jet is due to a single major factor. A X^2 test for goodness of fit to a 1:1 ratio gave a probability of 0.20 to 0.1o and hence agrees with the other tests which showed that Jet's resistance is due to a single dominant gene.

In the above conclusion arising from the F_2 backcross generation of Sanalta x F_1(Sanalta x Jet) it has been assumed that the group of 36 families falling in the low infection group came from heterozygous resistant plants. According to the above assumption this group should have an average infection rate similar to the F_2 generation of the cross Sanalta x Jet. The average rate of infection was 4.01 per cent and is, therefore, in good agreement with the average infection of the F_2 generation of Sanalta x Jet (Table III).

The highly infected lines of this same backcross generation are well within the range of infection of the more susceptible parent, Sanalta (Table I) and hence it is very likely that these families were produced by homozygous recessive plants.

Data for the F_2 of the backcross generation of the cross Montcalm x F_1 (Montcalm x Jet) are given in Table VI. A split in these lines occurs in the 37.5 to 52.5 per cent class dividing the progenies into two groups of 65 and 51 families. A 1:1 ratio in the above distribution would be ex-

pected if the resistance of Jet is conditioned by a single gene. A test of goodness of fit of these data to the 1:1 ratio gave a P value of 0.20 to 0.10, hence the assumption of a major gene for resistance in Jet is acceptable. As in the case of the cross Sanalta x F_1 (Sanalta x Jet) it is possible to further analyze the two groups of families in the F_2 backcross generations of Montcalm x F_1 (Montcalm x Jet)(Table VI). The low infection group was assumed to have come from segregating plants of the Montcalm x Jet hybrid and hence should have the same average infection as the F_2 generation of Montcalm x Jet. An infection rate of 4.13 per cent for this group is in good agreement with the average infection rate of 3.92 per cent for the F_2 generation of Montcalm x Jet.

The high infection group of the F_2 backcross generation of Montcalm x F_1 (Montcalm x Jet) has been assumed to have developed from homozygous susceptible plants of the hybrid Montcalm x Jet. This group fits into the infection range of the susceptible parent, Montcalm (Table I). Hence it is apparent that the classification assumed for the two groups in the distribution of the backcross progeny (Table VI) may be accepted.

(c) Inheritance of reaction to race Tr. in Titan hybrids

The data on the distribution of the F_3 progeny of the cross Sanalta x Titan are given in Table VI and graphically illustrated in Figure 3. A complete break in the curve in the 27.5 to 37.5 per cent infection range divides the progenies into two groups with 150 lines in the low infection class and 66 lines in the high infection class. This division of the progenies into two groups approximates a 3:1 ratio which would be expected if resistance of Titan to the Tr. were conditioned by a single dominant gene. A X^2 test for goodness of fit to a 3:1 ratio gave a probability of 0.10 to 0.05 and hence indicates that such a gene does exercise its influence in Titan.

An attempt to classify the F_3 progeny of the cross Sanalta x Titan into homozygous resistant, segregating, and homozygous recessive gave a distribution which did not conform to the 1:2:1 ratio that should exist if a single gene ~~was~~ were responsible for resistance of Titan. This situation is probably due to Titan not being a completely resistant variety (Table I) and is shown by a reduced number of F_3 lines in the 0 per cent infection class.

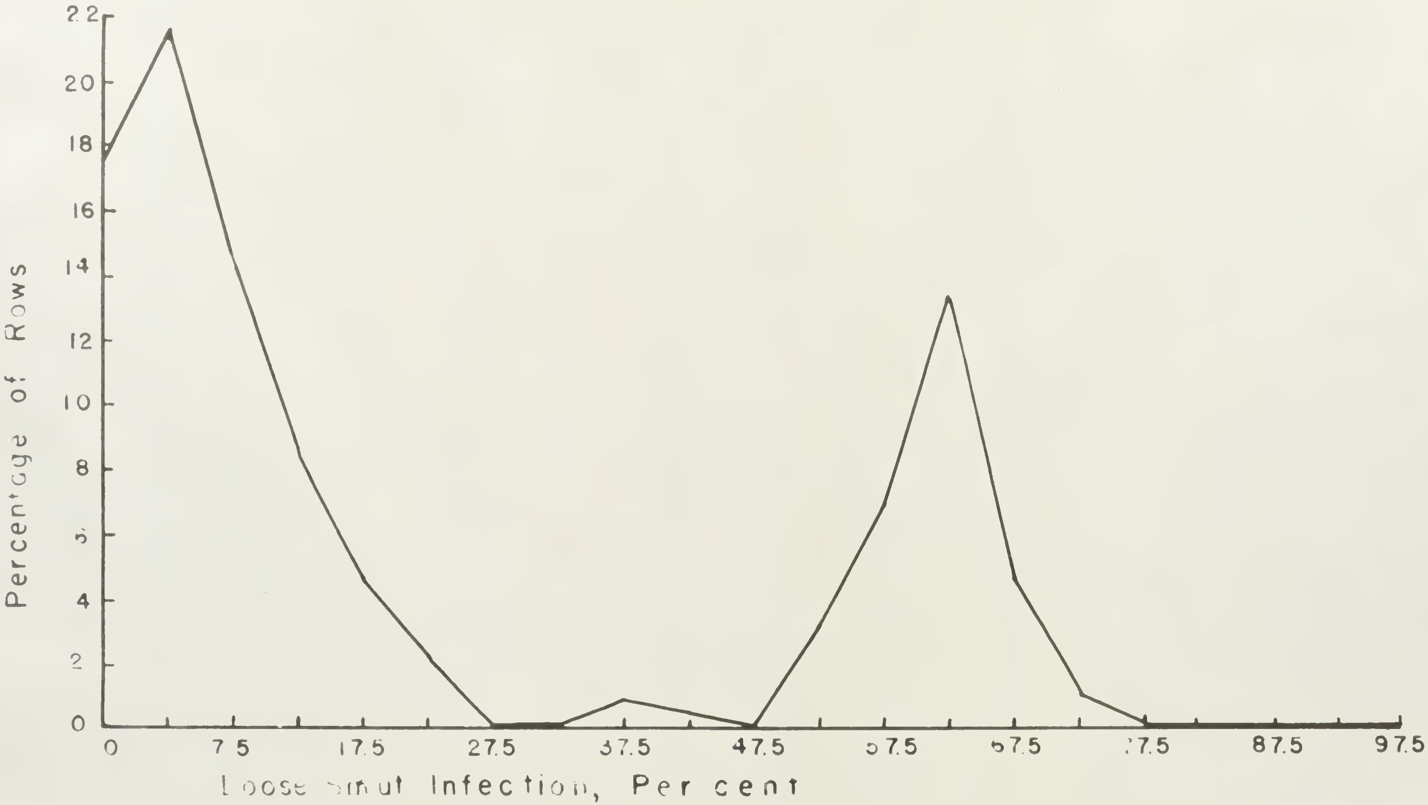

FIG. 3. Distribution of 216 F_3 lines of Sonalta x Titan inoculated with Ustilago nuda, race Tr.

The distribution of the F_3 progeny of the cross Montcalm x Titan is given in Table VI and illustrated graphically in Figure 4. The small number of lines in the 32.5 to 47.5 per cent class forms two groups with 131 lines showing an infection of 0 to 37.5 per cent and 35 lines in the 42.5 to 67.5 per cent class. As in the cross of Sanalta x Titan the above distribution approximates a 3:1 ratio and hence points to the presence of a single major gene for resistance to loose smut in Titan. The above distribution gave a X^2 test for a 3:1 ratio with a probability of 0.30 to 0.20.

As in the case of F_3 progeny of Sanalta x Titan there was no harmony with a 1:2:1 ratio when the distribution was classified as homozygous resistant (0 per cent infection), segregating (1 to 37.5 per cent infection) and homozygous susceptible (42.5 to 67.5 per cent infection). This is probably due to Titan being incompletely although highly resistant (Table I) and hence the 0 per cent infection class is low, (Table VI).

The data for the distribution of the F_3 progeny of the cross Titan x Montcalm is given in Table VI. The small number of lines in the 32.5 to 47.5 per cent infection class divides the families into two definite groups. Of the 184

FIG.4. Montcalm x Titan. Distribution of 166 F_3 lines inoculated with Ustilago nuda, race Tr.

F_3 lines, 142 fall in the 0 to 40 per cent infection range, while 42 are in the 40 to 77.5 per cent range. This distribution approximates a 3:1 ratio which would be the result of Titan having a single gene for resistance. In testing the goodness of fit of these data to the 1:1 ratio a P value of 0.50 to 0.30 was obtained.

It was also possible to classify this F_3 distribution further. Since Titan is almost entirely resistant (Table I) to race Tr. the progeny falling in the 0 per cent infection class may be considered to have come from homozygous resistant F_2 plants. Plants in the F_3 high infection group, 40.0 to 77.5 per cent, have a distribution that fits closely the infection range of the susceptible parent, Montcalm, and therefore, they may be assumed to have been produced by homozygous susceptible F_2 plants. The group in the 1 to 40 per cent infection range was assumed as coming from segregating F_2 plants of the cross Titan x Montcalm. This assumption is confirmed by the fact that its average infection of 7.03 per cent is in very good agreement with the average infection of the F_2 generation of the cross Montcalm x Titan.

According to the above classification there were 41 homozygous resistant F_3 lines, 100 segregating F_3 lines and 43 homozygous recessive F_3 lines.

The distribution of the F_3 progenies approximates a 1:2:1 ratio. A X^2 test for goodness of fit to the above ratio gave a probability of 0.50 to 0.30 and strongly suggests the operation of a single major gene for resistance to loose smut in Titan.

The Titan x Montcalm cross was the only reciprocal cross carried through to the F_3 generation. Results obtained from this cross are in good agreement with those obtained from Montcalm x Titan and therefore show that reaction of reciprocal crosses to infection by the loose smut organism is the same.

The data on the distribution of 64 backcross progeny of the cross Sanalta x F_1 (Sanalta x Titan) are given in Table VI. A break in the infection curve divides the lines into two groups with 26 lines in the 0 to 32.5 per cent infection range and 38 lines in the 42.5 to 67.5 per cent infection range. If resistance of Titan is due to a single gene, a 1:1 ratio would be expected in the above lines. A X^2 test for goodness of fit to a 1:1 ratio gave a P value of 0.50 to 0.30.

3. F_2 Data on Resistance to Race Ts.

(a) Reaction to race Ts.

The data for the infection of F_2 hybrids of the various crosses when inoculated with race Ts. are given in Table VII.

Due to reasons given previously, no attempt was made to give an analysis from the F_2 population of the number of factors involved in conditioning resistance to race Ts.

A very good agreement existed in the amount of infection observed in reciprocal crosses of Jet with Sanalta and Montcalm, as well as reciprocal crosses of Titan with Sanalta and Montcalm and Titan with Jet (Table VII).

Comparison of the infection rate of F_2 Jet hybrids inoculated with Tr. showed that a consistently higher rate of infection existed when the inoculum was Ts. Jet has been found to be entirely resistant to both races of loose smut (Tables I and II) and therefore, the above results were probably attributed to the greater susceptibility of Sanalta, Montcalm and Titan to Ts.

Infection of F_2 barley hybrids inoculated with chlamydospores of race Ts.

Hybrid	Total plants Number	Infected plants Number	Infected plants Per cent
Sanalta x Jet	310	19	6.13
Jet x Sanalta	292	24	8.22
Total	602	43	7.14
Montcalm x Jet	287	23	8.01
Sanalta x Titan	442	340	76.92
Titan x Sanalta	426	295	69.24
Total	868	635	73.16
Montcalm x Titan	465	241	51.83
Titan x Montcalm	392	263	64.54
Total	857	504	58.81
Titan x Jet	284	10	3.52
Jet x Titan	193	10	5.18
Total	477	20	4.19

F_2 hybrids involving Titan and Sanalta as well as Titan and Montcalm present a different picture. The average infection of the F_2 progenies of the cross Sanalta x Titan is 73.16 per cent (Table VII) which is closer to the infection rate of Sanalta (Table II). Titan appears to contribute only a slight degree of resistance to its hybrids and thus confirms the statement made regarding the F_1 generation involving Titan that it probably carries only a weak gene for resistance.

The striking point concerning Titan is that its resistance to Ts. has been lowered considerably whereas Jet remains as immune as it did to Tr. This consistency of resistance of Jet to the two races of loose smut and the shifting of Titan from a resistant to a susceptible variety suggests that different genes may operate producing resistance in Titan and Jet.

The reaction of the F_2 reciprocal crosses of Titan with Jet behave in accordance with the other hybrids in that the infection rate is slightly higher than in the F_1.

<u>(b) Inheritance of reaction to race Ts. in Jet hybrids.</u>

Jet has maintained its immunity to race Ts. as well as to race Tr. while Sanalta showed a higher susceptibility to the former race of loose smut.

The distribution of 184 F_3 progenies of the cross Sanalta x Jet is shown in Table VIII and graphically illustrated in Figure 1. The small number of lines in the 52.5 to 62.5 per cent infection range divides the families into two groups. There are 147 F_3 lines in the low infection group and 37 F_3 lines in the high infection group. This distribution suggests a 3:1 ratio which would exist if one major gene were in operation. A test for goodness of fit to a 3:1 ratio gave a P value of 0.20 to 0.10 and thus this behaviour of the F_3 progenies may be attributed to a single gene for resistance to Ts. in Jet.

Since Jet is immune to infection it may be concluded that all F_3 families without infection must have come from F_2 plants that had the same complement of genes as Jet, i.e., all lines with 0 per cent infection may be classed as homozygous resistant. F_3 lines in the high infection group of the cross Sanalta x Jet are in good agreement with the infection range of the susceptible variety, Sanalta, and therefore, are classified as homozygous susceptible. F_3 lines in the 1 to 52.5 per cent infection range may be assumed to have been produced by F_2 plants having a heterozygous complement of genes. An average infection of 6.08 per cent of this F_3 group is in good agreement with the average infection of the F_2 generation of the cross Sanalta

Table VIII

Distribution of F_3 and backcross progenies for loose smut infection by percentage classes for crosses indicated. Artificially inoculated with race Ts.

Crosses	No. of lines	Percentage classes 0	0.1-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	70-75	75-80-	80-85	86-90	90-95	95-100
Sanalta x Jet	184	52	36	16	14	8	7	5	3	1	1	3	1	0	0	2	0	3	11	18	1	2
Sanalta x F_1 (Sanalta x Jet)	108	0	36	16	3	0	2	3	1	0	0	0	0	0	8	3	3	10	22	0	1	
Montcalm x Jet	130	42	0	1	8	1	14	20	2	1	1	0	1	3	2	5	9	17	0	3	0	0
Montcalm x F_1 (Montcalm x Jet)	98	0	0	1	29	17	5	3	1	1	2	0	1	2	4	8	5	18	1	0	0	0
Titan x Jet	84	66	0	1	2	0	0	8	4	1	2	0	0	0	0	0	0	0	0	0	0	0

x Jet (Table VII) and thus justifies the classification of this group as the heterozygous.

According to the above classification there were 52 homozygous resistant F_3 lines, 95 segregating F_3 lines and 37 homozygous susceptible lines. This distribution indicates a 1:2:1 ratio which would be the case if one gene controlled resistance. A X^2 test for goodness of fit to the 1:2:1 ratio gave a probability of 0.30 to 0.20.

Data for the F_3 distribution of the cross Montcalm x Jet is given in Table VIII and graphically illustrated in Figure 2.

A small number of lines in the 32.5 to 57.5 per cent infection class divides the 130 F_3 progenies into two groups which give a satisfactory 3:1 ratio. There were 90 lines in the 0 to 42.5 per cent infection range and 40 lines in the 52.5 to 87.5 range. A X^2 test for goodness of fit to the 3:1 ratio gave a P value of 0.20 to 0.10 and therefore, it is concluded that Jet has a single major gene for resistance to *Ustilago nuda* Ts.

An attempt to classify further the progenies into homozygous resistant, segregating, and homozygous susceptible lines failed to show agreement with a 1:2:1 ratio when the X^2 test for goodness of fit was applied. This is probably due to the small number of lines in the population.

Data for the distribution of the F_2 backcross progenies of the cross Sanalta x F_1 (Sanalta x Jet) are given in Table VIII. There is a long break in the infection curve showing no smutted lines in the 32.5 to 62.5 per cent range. This division into two groups of 61 lines and 47 lines approximates a 1:1 ratio which would be expected in this case if a single gene for resistance were present in Jet.

The low infection group must have come from a heterozygous complement of genes of Sanalta and Jet and therefore, its average infection should be approximately equal to that of the F_2 generation of the cross involving Sanalta with Jet and inoculated with Ts. An average 6.02 per cent infection for this low infection backcross group is in good agreement with an average 7.14 per cent infection of the F_2. Sanalta x Jet cross (Table VII) so that classifying it as coming from segregating plants is justified. The high infection group of the backcross progeny of Sanalta x F_1 (Sanalta x Jet) fits well into the infection range of the susceptible parent, Sanalta, thus justifying the assumption that it was produced by homozygous susceptible plants. A X^2 test for goodness of fit to a 1:1 ratio gave a probability of 0.20 to 0.10, which strongly suggests the existence of a single major gene for resistance in Jet.

In the distribution of the backcross progenies of Montcalm x F_1 (Montcalm x Jet) (Table VIII), there was a sharp dip in the infection curve dividing the progenies into two groups. Of the 98 backcross lines studied, 59 lines were in the 7.5 to 42.5 infection class and 39 lines were in the 52.5 to 82.5 infection class. This distribution was not in agreement with a 1:1 ratio which would be expected if a single dominant gene conditioned the resistance of Jet to loose smut. However, the distribution of the F_3 progenies of Montcalm x Jet indicated so strongly that a single gene was responsible for Jet's resistance that the data on the distribution of the backcross progenies of Montcalm x F_1 (Montcalm x Jet) may be considered as not having been distributed normally.

The data for the F_3 progeny of Titan x Jet are given in Table VIII. Although the number of lines is only approximately half of that used in other hybrids, the distribution fits a 3:1 ratio remarkably well. Lines without any infection may be assumed to have come from F_2 plants that were homozygous resistant to ract Ts., i.e., had the same complement of genes as Jet for resistance. F_3 lines falling in the 7.5 to 42.5 infection group are within the infection range of the susceptible parent; Titan (Table II).

According to the above classification there were 66 homozygous resistant F_3 lines and 18 susceptible F_3 lines. In testing for a goodness of fit to a 3:1 ratio a P value of 0.95 to 0.50 was obtained. Such a ratio would be expected if a single major gene conditioned resistance to race Ts. in Jet.

GENERAL DISCUSSION

The inheritance of resistance to the brown loose smut of barley caused by Ustilago nuda, was studied in barley hybrids of Jet and of Titan. The F_1 generation usually equalled or approached the resistance of the more resistant parent. When floral inoculations were made on plants heterozygous for resistance, both resistant and susceptible segregates were obtained. This shows that the floral tissue was not responsible for the smut reaction of the plant. The seat of resistance, therefore, must lie in the embryo. The infection of F_1 plants of reciprocal crosses (Table IV) also suggests that the fungus reaches the embryo with approximately equal frequency, whether the female parent is resistant or susceptible.

The genetical analysis of Jet in this study showed it to be absolutely resistant to the Tr. and the Ts. races of Ustilago nuda. The heterozygous plants were equally as resistant as the parent. This high type of resistance in Jet was found in all cases to be due to a single dominant gene. Similar results were observed by Schaller (10).

Titan showed a high but not absolute resistance to race Tr. and this investigation showed that a single dominant

gene was responsible. The reaction of Titan to race Ts. was entirely different. Its resistance to this race broke down to the extent of relegating it to the susceptible class.

The breakdown of Titan's resistance to race Ts. and the maintenance of Jet's complete resistance to this new race may suggest that the genes for resistance in Titan and Jet were different. Schaller (10) suggested that there were some indications that the genes for Trebi and Jet were different. Titan, derived from Trebi, is considered to have the Trebi resistance and what is true for Trebi is probably also true for Titan. The writer feels, however, that the gene in Jet and in Titan conditioning resistance to race Tr. is the same. Jet is able to maintain its high resistance to race Ts. probably due to the operation of a supplementary gene which is absent in Titan and hence the difference in resistance to this new race of smut by the above mentioned varieties.

Both Sanalta and Montcalm showed a consistently higher degree of susceptibility when inoculated with Ts. as compared with Tr. In the distribution of the F_3 progenies of these varieties crossed with Jet or Titan there was a definite shift to a higher level of infection of the homozygous susceptible F_2 plants when race Ts. was used as inoculum. It is very likely that this condition prevails due to the greater virulence of the new race of smut, *Ustilago nuda* race Ts.

An inoculation method that will give 100 per cent infection with Ustilago nuda has not yet been devised. However, it was doubtful whether failure to secure this complete infection was really the fault of the technique. The degree of infection may be limited by the penetrance of each particular variety and although it is homozygous susceptible the average rate of incomplete infection will be maintained for each.

Artificial inoculation as outlined by Poehlman (8) has been used, but with a slight modification when this operation was performed in the greenhouse. Preliminary tests showed that there was a great deal of flower mortality after inoculation. Injection of the aqueous suspension of chlamydospores, followed by suction of excess water from the floret, resulted in a much higher seed set than when the floret was left full of water. Humidity conditions in the greenhouse are generally very high, and this coupled with absence of air currents resulted in a very slow evaporation of excess water from the florets resulting in failure to develop.

CONCLUSIONS

The high resistance of Jet and Titan to race Tr. is due to the operation of a single dominant gene. The existence of a new race of smut Ts., has been demonstrated. When artificially inoculated with Ts. Jet maintained its immunity but Titan behaved as a susceptible variety.

It is probable that resistance to Tr. in Jet and Titan is due to a single main gene and that Jet is able to exercise its full resistance to Ts. because of the presence of a supplementary gene, which is absent in Titan.

A six-rowed, white variety called Anoidium has been shown to be immune to both races of loose smut. It may be more practical to carry on any further genetical work on the inheritance of resistance to loose smut, *Ustilago nuda*, in barley by using this variety in place of Jet.

ACKNOWLEDGEMENTS

The author wishes to express his thanks and appreciation to Dr. L. P. V. Johnson, under whose supervision this study was conducted and the manuscript prepared; and to the National Research Council of Canada, for financial assistance in the form of a bursary.

REFERENCES

1. FREEMAN, E. M. and JOHNSON, E. C. The loose smuts of barley and wheat. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 152. 1909.

2. LIVINGSTON, J. E. The inheritance of resistance to _Ustilago nuda_. Phytopathology 39:959-979. 1949.

3. POEHLMAN, J. M. A simple method of inoculating barley with loose smut. Phytopathology 35:640-644. 1945.

4. ROBERTSON, D. W., WIEBE, G. A. and SHANDS, R. G. A summary of linkage studies in barley. Supplement I, 1940-1946. Jour. Amer. Soc. Agron. 39:464-473. 1947.

5. RODENHISER, H. A. Physiologic forms of _Ustilago nuda_ and _Ustilago tritici_. Phytopathology 16:1001-1007. 1926.

6. ________________ Physiologic specialization in some cereal smuts. Phytopathology 18:955-1003. 1928.

7. ROSS, J. G., SEMENIUK, W., TAYLOR, D. K. and JENKINS, B. C. Factors affecting the degree of infection of barley by loose smut (_Ustilago nuda_ (Jens.) Rostr.) Sci. Agric. 28:481-492. 1948.

8. RUTTLE, Mable L. (Mrs. Nebel). Studies on barley smuts and on loose smut of wheat. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bull. 221. 1934.

9. SCHAFER, L. A. and HANSING, E. D. Effect of hot-water treatment on emergence of spring barley and control of brown loose smut (_Ustilago nuda_). Phytopathology 40:518-521. 1950.

10. SCHALLER, C. W. Inheritance of resistance to loose smut, _Ustilago nuda_, in barley. Phytopathology 39: 959-979. 1949.

11. SEMENIUK, W. and ROSS, J. G. Relation of loose smut to yield of barley. Can. Jour. Research (C.) 20:491-500. 1942.

12. SHANDS, H. L. and SCHALLER, C. W. Response of spring barley varieties to floral loose smut inoculation. Phytopathology 36:534-548. 1946.

13. THREN, R. The question of the physiological specialization of loose smut of barley, Ustilago nuda (Jensen) Kellerm et Sw. and the origin of new barley smut races. Phytopathology Z. 13: 539-571. 1941.

14. WELLS, S. A. and PLATT, A. W. The effect of loose smut on the viability of artificially inoculated barley seeds. Sci. Agric. 29:45-52. 1949.

15. ZEINER, W. Das Verhalten verschiedener Sommergerstenkreuzungen hinsichtlich der Anfälligkeit für Ustilago nuda. Ztschr. f. Züchtung, A. 17: 229-264. 1932.